Safety Assessment of Glycerin as Used in Cosmetics

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All interested persons are provided 60 days from the above date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian J. Gill P.A.

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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INTRODUCTION

This is a review of the available scientific literature and unpublished data provided by industry relevant to assessing the safety of glycerin as used in cosmetics. Glycerin is reported to function in cosmetics as a denaturant; fragrance ingredient; hair conditioning agent; humectant; oral care agent; oral health care drug; skin protectant; skin-conditioning agent - humectant; and viscosity decreasing agent.[1]

CHEMISTRY

Definition and Structure

Glycerin (CAS No. 56-81-5) is the polyhydric alcohol that conforms generally to the structure in Figure 1.[1] The molecular formula is $C_3H_8O_3$. Glycerin (also referred to as glycerol in the literature) is a simple polyol compound that has three hydroxyl groups.

Glycerin is naturally occurring in all animals and plant matter in combined form as glycerides in fats and oils, or, intracellular spaces as lipids.[2]

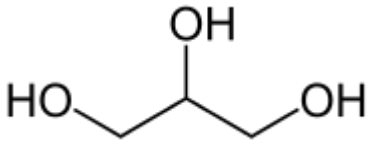

Figure 1. Glycerin

Physical and Chemical Properties

Chemical and physical properties are provided in Table 1.

Glycerin is seldom in its crystallized state due to its tendency to supercool, and to the pronounced effect of small amounts of water in depressing the melting (freezing) point.[2]

Glycerin has solvent properties, similar to those of water and simple aliphatic alcohols, due to its 3 hydroxyl groups.[3] It is completely miscible with water, methanol, ethanol, and the isomers of propanol, butanol, and pentanol. Glycerin is also fully miscible with phenol, glycol, propanediols, amines, and heterocyclic compounds containing a nitrogen atom in the ring (e.g., pyridine, quinoline). It has less solubility in acetone, diethyl ether, and dioxane. Glycerin is almost insoluble in hydrocarbons, long-chain aliphatic alcohols, fatty oils, and halogenated solvents such as chloroform.

Glycerin decomposes to di/polyglycolethers and acrolein at >180˚C. In a closed bottle biodegradation test (performed according to OECD 301) 92% biodegradation was reported after 30 days. At 10 days, more than 60% biodegradation was observed (measured as theoretical oxygen demand).[2,4]

Method of Manufacture

The starting material for synthetic glycerin may be allyl chloride, acrolein, propylene oxide, sugar, polyalcohols, fats, or epichlorohydrin.[3] Natural or native glycerin is obtained as a byproduct in the conversion of fats and oils to fatty acids or fatty acid methyl esters.

Presented here are two representative examples of the several methods to manufacture glycerin.

Allyl chloride is oxidized with hypochlorite to dichlorohydrin, which is converted without isolation to epichlorohydrin by ring closure with calcium or sodium hydroxide.[3] Epichlorohydrin is hydrolyzed to glycerin at 80-200˚C with a 10%-15% aqueous solution of sodium hydroxide or sodium carbonate at atmospheric or overpressure. The yield of dilute (10%-25%) glycerin solution is >98%, and it contains 5%-10% sodium chloride and <2% of other impurities. This aqueous glycerin solution is evaporated in a multistage evaporation plant under vacuum to a glycerin concentration of >75%, separating precipitated sodium chloride. The glycerin solution is then distilled under high vacuum; co-distilled water is separated by fractional condensation. The glycerin is treated further to remove color impurities and odorous material; this can be performed, for example, with activated carbon.

Propene is oxidized to acrolein, which is then reduced by Meerwein-Ponndorf-Verley to allyl alcohol.[3] The allyl alcohol is epoxidized with hydrogen peroxide, and the resulting glycidol is hydrolyzed to glycerin.

Impurities

Glycerin is reported to be 95%-99.5% pure.[4] Impurities are water and trace levels of polyglycerol.

USE

Cosmetic

The Food and Drug Administration (FDA) collects information from manufacturers on the use of individual

ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). Glycerin is reported to be used in 15 654 cosmetic products; 10 046 are leave-on products, 5441 are rinse-off products, and 167 products are diluted for the bath. These uses include 862 eye products, 160 lipsticks, 369 hair dyes and colors, 1259 bath soaps and detergents, 7756 skin care products, and 244 suntan preparations (Table 2).[5]

A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for ingredients in this group. Glycerin is reported to be used at up to 78.5% in leave-on products, 68.6% in rinse-off products, and up to 47% in products diluted for the bath. It is used at up to 21% in baby products, up to 40.6% in eye lotion, 25% in perfumes, 47.3% in hair grooming aids, 68.6% in oral hygiene products, 78.5% in body and hand skin care products, 17.9% in suntan preparations.[6]

Industry is not required to register products with the VCRP. It is understood that the data in the database are a sampling of what cosmetics are available on the market and are not comprehensive. Not all cosmetic manufacturers are members of the Council and not all members respond to the survey request. It is understood that the information collected are also not comprehensive but do represent a large sampling of cosmetic products.

Glycerin was reported to be used in aerosol/spray products that include: hair sprays (in aerosols up to 10% and pump sprays up to 30%), spray deodorants up to 2%, face and neck products up to 10%, body and hand products up to 5%, moisturizing products up to 3.3%, and suntan products (in aerosols up to 6% and pump sprays up to 10%). These aerosol/spray products could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.[7-10] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[7,9] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[7] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Non-Cosmetic

Glycerin is considered generally recognized as safe (GRAS) by the FDA for food packaging and as a multiple purpose food substance.[21CFR182.90; 21CFR182.132] Glycerin is also used as an active ingredient in over-the-counter drugs (Table 3).

Glycerin has been administered orally and/or intravenously to reduce intracranial pressure due to various medical conditions.[11] Glycerin has been used to reduce brain volume for neurosurgical procedures. It is also used as a laxative.

Glycerin functions as a humectant, solvent, cake icing component, confectionary component, bodying agent, and plasticizer for foods.[2]

Glycerin is used in paints, lacquers, and varnishes; polymers; tobacco; absorbents and adsorbents; adhesives and binding agents; anti-freezing agents; cleaning agents and disinfectants; explosives; heat transferring agents; pesticides; and softeners.[2] It is an intermediate and monomer in resins, polyols, and polyurethanes.[4]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Glycerin is rapidly absorbed in the intestine and the stomach, distributed over the extracellular space and excreted.[4,12,13] Glycerin is phosphorylated to α-glycerophosphate by glycerol kinase, predominantly in the liver (80%-90%) and kidneys (10%-20%), and incorporated in the standard metabolic pathways to form glucose and glycogen.[12,14] Glycerin kinase is also found in intestinal mucosa, brown adipose tissue, lymphatic tissue, lung and pancreas. Glycerin may also combine with free fatty acids in the liver to form triglycerides (lipogenesis) and distributed to adipose tissues. The turnover rate is directly proportional to plasma glycerol levels.[15]

Free glycerin is naturally present in human plasma.[4] Its excretion in the urine stops if levels fall below 1 mg/mL plasma.

Dermal/Percutaneous

Data on dermal absorption, distribution, metabolism, and excretion of glycerin were not found in the published literature nor were unpublished data provided.

Oral

Orally administered glycerin is rapidly absorbed from the gastrointestinal tract, and peak serum concentrations occur within 60-90 min.[11] Glycerin is distributed throughout the blood. Glycerin generally does not appear in ocular fluids; however, it may enter the orbital sac when the eye is inflamed. It is not known if glycerin is distributed into milk.

The elimination half-life of glycerin is approximately 30-45 min. Most orally administered glycerin is incorporated into body fat, metabolized by glycerokinase, principally in the liver to carbon dioxide and water, or is utilized in glucose or glycogen synthesis. Glycerin can also combine with free fatty acids to form triglycerides. Approximately 80% of glycerin metabolism takes place in the liver and approximately 10%-20% in the kidney. The metabolism of glycerin to carbohydrate

produces 4.3 calories/g glycerin. Most of an oral dose of glycerin is been metabolized within 2.5 h. Approximately 7%-14% of an oral dose of glycerin is excreted unchanged in urine during this time period.

Orally administered glycerin elevates the osmotic pressure of the plasma to such an extent that water from the extravascular spaces is drawn into the blood. The osmotic effect of glycerin produces a decrease in intraocular pressure (IOP) by reducing the volume of intraocular fluids in a manner completely independent of the normal ocular fluid inflow and outflow mechanisms. The extent of IOP reduction varies with the dose of glycerin and the etiology and degree of the increased pressure. Reduction in IOP reaches its maximum within 30 min to 2 h and may persist for 4-8 h. In general, reduction in IOP is greatest when pretreatment intraocular pressure is high. The osmotic effect of glycerin may also produce tissue dehydration and a decrease in cerebrospinal fluid pressure. Glycerin produces only very slight diuresis in healthy individuals receiving a single dose of 1.5 g/kg or less.[11]

Acute ingestion of glycerin (1 mL/kg in water) in male subjects led to an increase in plasma glycerides. In female subjects, the oral administration of glycerin (1 mL/kg in water) resulted in no change in plasma glyceride concentration. When glycerin (1 mL/kg/d in 3 doses) was orally administered for 42 days, increased serum glyceride concentrations were observed in both sexes, however, the increase was greater in men.[16]

TOXICOLOGICAL STUDIES

Acute Toxicity

Non-Human

The reported oral LD_{50} of glycerin in rats ranged from 2.530-27.200 g/kg, 4.090-37.763 g/kg in mice, 27.000 g/kg in rabbits, and from 7.750-77.500 g/kg in guinea pigs (Table 4).[2,14,17-23] The dermal LD_{50} of glycerin in rats was reported to be >21900 mg/kg and 18700 mg/kg for rabbits.[2,17] The intraperitoneal LD_{50} of glycerin in rats ranged from 4420-10100 mg/kg and 8600-9500 mg/kg for mice.[2] The subcutaneous LD_{50} of glycerin in rats was 100 mg/kg and ranged from 91-10000 mg/kg for mice.[2,23] The intravenous LD_{50} of glycerin in rats ranged from 5200-76600 mg/kg.[2]

Oral – Human

The LD_{LO} of glycerin was reported to be 1428 mg/kg for humans.[2]

There were no signs of toxicity when subjects (n=10 men, 4 women) were administered glycerin (30 mL; 95% in orange juice) after each of the 3 daily meals (assumed for 1 day).[4]

Adverse effects following the oral administration of glycerin include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea.[11] Headache may result from cerebral dehydration, which may be prevented or relieved by having the subject lie down during and after treatment. Hypotonic fluids will relieve thirst and headache caused by the dehydrating action of glycerin.

Repeated Dose Toxicity

Oral – Non-Human

The oral lowest observable adverse effects level (LOAEL) for glycerin was 950 mg/kg for rats for 3 days (Table 5).[24] In short-term feeding experiments, glycerin at 20% for 4 weeks in feed had no adverse effects but at 53.4%, the kidneys had increased weights and livers had increased enzyme activity.[25,26] The no observed adverse effects level (NOAEL) was between 115 and 2300 mg/kg when administered in water for 44 days.[27] Calcified masses were observed in kidney tubulus between cortex and medulla in 3 of 5 rats administered both natural and synthetic glycerin (3335 mg/kg) in drinking water for 6 months.[22] The NOAEL was approximately 10 000 mg/kg/d for rats when administered in the diet for 2 years.[17]

The no observable effect level (NOEL) for mongrel dogs was 950 mg/kg/d when orally administered for 3 days (Table 5). At 3800 mg/kg/d, the mucosa of the stomach was severely hyperemic with petechial hemorrhages.[28] Mongrel dogs experienced weight loss after 36 weeks when glycerin (35%) was incorporated in their feed. The weight loss continued when the glycerin content was reduced by 50%-80% for the remainder of a 50-week study.[24]

There were no pathological changes in guinea pigs (n=10) orally administered glycerin (6300 mg/kg/day) for 30-40 days (Table 5).[29]

Oral – Human

There were no signs of toxicity or effects on blood and urine production when subjects (n = 10 male, 4 female) were orally administered glycerin (~1.3 to ~2.2 g/kg/d; glycerin in orange juice with meals) for 50 days.[2] The NOAEL was ≥ 2200 mg/kg/d. No further information was provided.

There were no adverse effects observed in subjects (n=14) administered glycerin (30 ml, neat) 3 times daily with each meal for 50 days.[12]

There were no toxic effects to subjects (n=37) with cerebral edema caused by acute cerebral infarction who were orally administered glycerin (1.5 g/kg) for 4 days.[2] A second group of subjects (n = 17) with edema of the central nervous system were similarly treated. Mortality of the group with cerebral infarction was 11%, but was not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of treatment.

Dermal – Non-Human

There were no treatment effects when glycerin (100%; 0.5-4 mL) was administered to 30% of the body surfaces of rabbits for 45 weeks (Table 5).[17]

Inhalation – Non-Human

The inhalation LOAEL was 1000 mg/m^3 for glycerin administered 6 h/day, 5 days/week for 2 weeks in Crl:DCD Sprague-Dawley rats based on local effects on the epithelium of the upper respiratory tract (Table 5).[22,30]

The inhalation NOAEL was 0.167 mg/L for glycerin administered for 5 h/day, 5 days/week for 13 weeks in Crl:DCD Sprague-Dawley rats (Table 5).[22] There was minimal squamous metaplasia of the epiglottis in 2/25, 1/19, 4/20 and 10/21 rats at 0, 33, 167 and 662 mg/m^3, respectively; 1 male in the high-dose group showed mild squamous metaplasia.

Intravenous - Human

There were no toxic effects to subjects (n=37) with cerebral edema caused by acute cerebral infarction who were intraveneously administered glycerin (1.2 g/kg) for 4 days.[31] A second group of subjects (n=17) with edema of the central nervous system were similarly treated. Mortality of the group with cerebral infarction was 11%, but was not related to glycerin administration. Neurological improvement occurred in all other cases during and at the end of treatment.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a two-generation reproductive study of rats, (n=10 females), the administration of glycerin (0, 20%; ~ 2 g/kg/d in drinking water) for 12 weeks (8 weeks before mating until weaning of pups) no adverse effects were observed on the reproductive efficiency of the parents, nor on the growth, fertility, or reproductive performance of the untreated F1 generation.[4] No histological changes occurred in the tissues of either the F1 or F2 generations. The onset of estrus cycles, weight gain, and microscopic examination of the endocrine organs were comparable to control values for both F1 and F2 generations. In the parent generation all 10 females became pregnant with similar litter size as controls (9.0 vs. 8.1). In the F1 generation, 9 of 10 females became pregnant.

When glycerin (13.1, 60.8, 282 and 1310 mg/kg/d) was administered by gavage to Wistar rats (n=25-28) on days 6 through 15 of gestation, there were no adverse effects in the dams.[32] The NOAEL for maternal toxicity and teratogenicity was 1310 mg/kg/d. The number of pregnancies was: 23 of 25, 24 of 25, 22 of 28, 22 of 25, and 21 of 25 for controls, and at doses of 13.1, 60.8, 282 and 1310 mg/kg, respectively. The number of implantations, resorptions, litter sizes, weights, and sex ratio were similar among groups as were external, visceral, and skeletal abnormalities.

When glycerin (12.8, 59.4, 276 and 1280 mg/kg/d) was administered by gavage to CD-1 mice (n=25) on days 6 through 15 of gestation, there were no adverse effects in the dams.[32] The NOAEL for maternal toxicity and teratogenicity was 1280 mg/kg/d. The number of pregnancies was: 14/15, 12/15, 10/18, 13/20 and 13/15 for controls, and at doses of 12.8, 59.4, 276 and 1280 mg/kg, respectively. The number of implantations, resorptions, litter sizes, weights, and sex ratio were similar among groups as were external, visceral, and skeletal abnormalities.

When glycerin (11.8, 54.8, 254.5 and 1180 mg/kg/d) was administered by gavage to Dutch-belted rabbits (n=25) on days 6 through 18 of gestation, there were no adverse effects in the dams.[32] The NOAEL for maternal toxicity and teratogenicity was 1180 mg/kg/d. The number of pregnancies was: 22 of 25, 23 of 25, 20 of 25, 22 of 25 and 21 of 25 for controls, and at doses of 11.8, 54.8, 254.5 and 1180 mg/kg, respectively. The number of implantations, resorptions, litter sizes, weights, and sex ratio were similar among groups as were external, visceral, and skeletal abnormalities.

Male Fertility – Non-Human

Glycerin injected into the testes of rats (50-200 µL and 862 mg/kg) and monkeys (119 mg/kg) suppressed spermatogenesis (Table 6).[33-35]

Male Fertility – Human

In a fertility study of male employees (n=64) who manufacture glycerin, there were no differences observed in sperm counts and percent normal forms compared with a control group (n=63) who did not work with glycerin (Table 6).[36]

GENOTOXICITY

In Vitro

Glycerin was not genotoxic in multiple Ames tests using multiple strains of *Salmonella typhimurium* up to 50 mg/plate (Table 7).[19,37-41] It was not genotoxic in a cytogenetic assay, X-linked hypoxanthine-guanine phosphoribosyl transferase (hgprt), a sister chromatid exchange assay, unscheduled DNA synthesis assay, and a chromosome aberration test up to 1.0 mg/mL.[37,39]

In Vivo

In two chromosome aberration assays, glycerin was not genotoxic when administered orally to rats at 1 mg/kg or by injection into the abdomen at 1 g/kg (Table 7).[42] Glycerin had ambiguous results in an inhibition of DNA synthesis assay at 18.4 g/L. In a dominant lethal gene assay, there were ambiguous results.

CARCINOGENICITY

Glycerin administered in the feed of rats at doses up to 10 g/kg for 2 years and up to 20% for 1 year did not increase the incidence of tumors (Table 8).[17] Glycerin administered in drinking water, up to 5%, had a synergistic effect with 4-nitroquinoline 1-oxide (4NQO) in mice.[43-45]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

Glycerin was not dermally irritating to rabbits at concentrations up to 100% (Table 9).[17,19,46] Glycerin was a mild dermal irritant at 100% in guinea pigs.[23]

Dermal – Human

Glycerin (50% in water) was not irritating to subjects with dermatitis (n = 420) when administered for 20-24 h under occlusion.[47] One subject had a positive reaction. She reported using a mixture of glycerin (1 part) and 70 % ethanol (9 parts) applied on the hands after washing with soap and water. She was tested with glycerin (1%, 5%, 10% in water) and this glycerin-ethano1mixture (100%) resulting in +++ reactions for both test substances at 48 and 72 h.

Glycerin (10%; 0.05 mL) was slightly irritating in a 48-h occlusive patch test.[2] The irritation score was 4 out of 9 on day 14 of observation. No further information was provided.

Ocular -- Non-Human

Glycerin was not irritating to the eyes of rabbits at concentrations up to 100% (Table 10).[17,19,23,46]

Ocular -- Human

Topical administration of anhydrous glycerin in the eyes of human subjects with edema of the superficial layers of the cornea resulted in reduced edema and improved visualization.[11] Pain and/or irritation have occurred following administration of glycerin to the eye.

Glycerin (100%) was not irritating when administered to the eyes of human subjects (n not specified).[2] There was a strong burning and stinging sensation, with tear production, but no injury was observed.

Sensitization

Dermal – Non-Human

Both natural and synthetic glycerin were not sensitizing to white male guinea pigs (n=12).[17] The induction was 10 injections of 0.1 mL of a 0.1% solution every other day. The challenge was an injection of 0.05 mL of the 0.1% solution after a 2 week rest.

Dermal – Human

Subjects (n=15) that worked in a foam rubber factory that were regularly exposed to glycerin were not sensitized to glycerin (concentration not specified; in water) when patched tested at 100% for 48 h.[48]

SUMMARY

This is a safety assessment of glycerin, a polyhydric alcohol. Glycerin is reported to function in cosmetics as a denaturant; fragrance ingredient; hair conditioning agent; humectant; oral care agent; oral health care drug; skin protectant; skin-conditioning agent - humectant; and viscosity decreasing agent.

Impurities were reported to be water and trace levels of polyglycerol.

Glycerin is reported to be used in 15 654 cosmetic products; 10 046 are leave-on products, 5441 are rinse-off products, and 167 are products that are diluted for the bath. Glycerin is reported to be used up to 78.5% in leave-on products, 68.6% in rinse-off products, and up to 47% in products diluted for the bath.

Glycerin is considered to be GRAS by the FDA for food packaging and as a multiple purpose food substance. Glycerin is also used as an active ingredient in over-the-counter drugs.

Glycerin is rapidly absorbed in the intestine and the stomach, distributed over the extracellular space and excreted. Free glycerin is naturally present in human plasma.

The oral LD_{50} for rats ranged from 2.530-27.200 g/kg, 4.090-37.763 g/kg for mice, 27.000 g/kg for rabbits, and 7.750-77.500 g/kg for guinea pigs. The dermal LD_{50} for rats was reported to be >21.900 g/kg and 18.700 g/kg for rabbits. The intraperitoneal LD_{50} for rats ranged from 4.420-10.100 g/kg and 8.600-9.500 g/kg for mice. The subcutaneous LD_{50} for rats was 100 mg/kg and ranged from 0.091-10.000 g/kg for mice. The intravenous LD_{50} for rats ranged from 5.200-76.600 g/kg.

The LD_{LO} of glycerin was reported to be 1428 mg/kg for humans. There were no signs of toxicity when human subjects were administered 30 mL glycerin. Adverse effects to human subjects following the oral administration of glycerin include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea.

The oral LOAEL for glycerin was 950 mg/kg for rats for 3 days. In short-term feeding experiments using rats, 20% glycerin for 4 weeks in feed had no adverse effects but at 53.4%, the kidneys had increased weights and livers had increased enzyme activity. The NOAEL was approximately 10.000 g/kg/d for rats when administered in the diet for 2 years. The NOEL for mongrel dogs was 950 mg/kg/d when orally administered for 3 days. At 3800 mg/kg/d, the mucosa of the stomach was severely hyperemic with petechial hemorrhages. Mongrel dogs experience weight loss after 36 weeks when 35% glycerin was incorporated in their feed. There were no pathological changes in guinea pigs orally administered 6300 mg/kg/day glycerin for 30-40 days.

There were no signs of toxicity or effects on blood and urine production when human subjects were orally administered approximately 1.3-2.2 g/kg/d glycerin for 50 days. The NOAEL was ≥2200 mg/kg/d.

There were no treatment effects when 100% glycerin was administered to 30% of the body surfaces of rabbits for 45 weeks.

The inhalation LOAEL was 1000 mg/m^3 for glycerin administered 6 h/day, 5 days/week for 2 weeks in rats. The inhalation NOAEL was 0.167 mg/L for glycerin administered for 5 h/day, 5 days/week for 13 weeks in rats.

There were no toxic effects to subjects with cerebral edema who were intraveneously administered glycerin 1.2 g/kg for 4 days.

No adverse effects were observed in rats administered 20% glycerin in drinking water throughout gestation and nursing of pups. F1 generation reproduced normally. The oral NOAEL for maternal toxicity and teratogenicity for rats was 1310 mg/kg/d. The NOAEL for maternal toxicity and teratogenicity for mice was 1280 mg/kg/d. The NOAEL for maternal toxicity and teratogenicity for rabbits was 1180 mg/kg/d.

Glycerin injected into the testes of rats (50-200 µL and 862 mg/kg) and monkeys (119 mg/kg) suppressed spermatogenesis.

Glycerin was not genotoxic in multiple Ames tests using multiple strains of *S. typhimurium* up to 50 mg/plate. It was not genotoxic in a cytogenetic assay, X-linked hgprt, a sister chromatid exchange assay, unscheduled DNA synthesis assay, and a chromosome aberration test up to 1.0 mg/mL.

In two chromosome aberration assays, glycerin was not genotoxic when administered orally to rats at 1 mg/kg or by injection into the abdomen at 1 g/kg. Glycerin had ambiguous results in an inhibition of DNA synthesis assay at 18.4 g/L. In a dominant lethal gene assay, there were ambiguous results.

Glycerin administered in the feed of rats at doses up to 10 g/kg for 2 years and up to 20% in feed for 1 year did not increase the incidence of tumors. Orally administered glycerin, up to 5%, had a synergistic effect with 4NQO in mice.

Glycerin was not dermally irritating to rabbits when up to 100% glycerin was administered on up to 30% of the body surface for 8 h/day, 5 days/week for 45 weeks. Glycerin was a mild dermal irritant at 100% in guinea pigs.

Glycerin at 50% was not irritating to subjects with dermatitis.

Undiluted glycerin was not irritating when administered to the eyes of human subjects. There was a strong burning and stinging sensation, with tear production but no injury was observed.

Both natural and synthetic glycerin was not sensitizing to white male guinea pigs at 0.1%.

DATA NEEDS

The CIR staff request the following information:

- Manufacturing process used for cosmetic grade glycerin
- Dermal and inhalation absorption and metabolism
- Any toxicity data developed in the last 20 years

TABLES

Table 1. Chemical and physical properties of glycerin.

Property	Value	Reference
Physical Form	Liquid, syrupy	[4,49]
Color	Clear	[49]
Odor	Odorless, mild	[3]
Molecular Weight g/mol	92.09	[50]
Density/Specific Gravity @ 20ºC	1.26	[4]
Viscosity kg/(s m)@ ºC	1.41	[4]
Vapor pressure mmHg@ 50ºC	0.0025	[49]
Vapor Density mmHg	3.17	[49]
Melting Point ºC	18	[4]
	17.9	[49]
Boiling Point ºC	290	[4]
Water Solubility	Miscible	[4]
log K_{ow}	1.76	[4]
Disassociation constants (pKa, pKb) @ºC	$0.07E^{-13}$	[4]

Table 2. Frequency of use according to duration and exposure of glycerin.[5,6]

Use type	Uses	Maximum Concentration (%)
Total/range	**15 654**	**0.0001-78.5**
Duration of use		
Leave-on	10 046	0.0001-78.5
Rinse-off	5441	0.0007-68.6
Diluted for (bath) use	167	0.66-47
Exposure type[a]		
Eye area	862	0.025-40.6
Incidental ingestion	353	2-68.6
Incidental Inhalation-sprays	6984[a]	0.075-77.3[b]; spray: 0.006-10, pump: 0.11-30
Incidental inhalation-powders	5859[c]	1-77.3[c]; powder: 0.024-15
Dermal contact	12 710	0.006-78.5
Deodorant (underarm)	136	Not spray: 0.1-10.4; pump: 2
Hair-noncoloring	1911	0.015-47.3
Hair-coloring	490	0.0007-20
Nail	5[7]	0.0001-45
Mucous Membrane	2597	0.66-68.6
Baby	125	2-21

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.

[a] Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

[b] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 3. FDA regulations on glycerin.

Citation	Regulation
	Food additive
21CFR172.866	FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION Synthetic glycerin produced by the hydrogenolysis of carbohydrates may be safely used in food, subject to the provisions of this section: (a) It shall contain not in excess of 0.2 percent by weight of a mixture of butanetriols. (b) It is used or intended for use in an amount not to exceed that reasonably required to produce its intended effect.
	Indirect food additive
21CFR175.300	INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS Resinous and polymeric coatings may be safely used as the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, in accordance with the following prescribed conditions: (a) The coating is applied as a continuous film or enamel over a metal substrate, or the coating is intended for repeated food-contact use and is applied to any suitable substrate as a continuous film or enamel that serves as a functional barrier between the food and the substrate. The coating is characterized by one or more of the following descriptions: (1) Coatings cured by oxidation. (2) Coatings cured by polymerization, condensation, and/or cross-linking without oxidation. (3) Coatings prepared from prepolymerized substances. (b) The coatings are formulated from optional substances that may include: (1) Substances generally recognized as safe in food. (2) Substances the use of which is permitted by regulations in this part or which are permitted by prior sanction or approval and employed under the specific conditions, if any, of the prior sanction or approval. (3) Any substance employed in the production of resinous and polymeric coatings that is the subject of a regulation in subchapter B of this chapter and conforms with any specification in such regulation. Substances named in this paragraph (b)(3) and further identified as required: (*b*) Rosin esters formed by reacting rosin (paragraph (b)(3)(v)(*a*) of this section) with: Glycerol (*c*) Polyhydric alcohols: Glycerol Isophthalyl dihydrazide for use only in coatings subject to the provisions of paragraph (c) (3) or (4) of this section. Trimellitic anhydride adducts of ethylene glycol and glycerol, prepared by the reaction of 1 mole of trimellitic anhydride with 0.4-0.6 mole of ethylene glycol and 0.04-0.12 mole of glycerol, for use only as a cross-linking agent at a level not to exceed 10 percent by weight of the cured coating, provided that the cured coating only contacts food containing not more than 8 percent alcohol. Epoxidized soybean oil (iodine number maximum 14; oxirane oxygen content 6% minimum), as the basic polymer. Glycerol (ii) Reconstituted oils from triglycerides or fatty acids derived from the oils listed in paragraph (b)(3)(i) of this section to form esters with: (iv) Natural fossil resins, as the basic resin: Glycerol ester of damar, copal, elemi, and sandarac. (v) Rosins and rosin derivatives, with or without modification by polymerization, isomerization, incidental decarboxylation, and/or hydrogenation, as follows: (*b*) Rosin esters formed by reacting rosin (paragraph (b)(3)(v)(*a*) of this section) with: Glycerol. (*c*) Polyhydric alcohols: Glycerol.
21CFR178.3500	INDIRECT FOOD ADDITIVES: ADJUVANTS, PRODUCTION AIDS, AND SANITIZERS Synthetic glycerin may be safely used as a component of articles intended for use in packaging materials for food, subject to the provisions of this section: (a) It is produced by the hydrogenolysis of carbohydrates, and shall contain not in excess of 0.2 percent by weight of a mixture of butanetriols. (b) It is used in a quantity not to exceed that amount reasonably required to produce its intended physical or technical effect, and in accordance with any limitations prescribed by applicable regulations in parts 174, 175, 176, 177, 178 and 179 of this chapter. It shall not be intended to, nor in fact accomplish, any direct physical or technical effect in the food itself.
21CFR182.90	SUBSTANCES GENERALLY RECOGNIZED AS SAFE Substances migrating to food from paper and paperboard products. Substances migrating to food from paper and paperboard products used in food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act, are as follows: Glycerin
21CFR182.1320	SUBSTANCES GENERALLY RECOGNIZED AS SAFE Subpart B--Multiple Purpose GRAS Food Substances (a)*Product.* Glycerin. (b)*Conditions of use.* This substance is generally recognized as safe when used in accordance with good manufacturing practice.
	Drug
21CFR310.545	NEW DRUGS Subpart E--Requirements for Specific New Drugs or Devices (10)External analgesic drug products --(i)Analgesic and anesthetic drug products. (vii)Poison ivy, poison oak, and poison sumac drug products. Glycerin (15)Topical otic drug products --(i)For the prevention of swimmer's ear and for the drying of water-clogged ears, approved as of May 7, 1991 . Acetic acid (ii)For the prevention of swimmer's ear, approved as of August 15, 1995. Glycerin and anhydrous glycerin

	(B)Ingredients--Approved as of June 4, 2004; June 6, 2005,for products with annual sales less than $25,000 . (iii)Diaper rash drug products. Glycerin (vi)Poison ivy, poison oak, and poison sumac drug products --(A)Ingredients--Approved as of November 10, 1993. Glycerin (vii)Poison ivy, poison oak, and poison sumac drug products. Glycerin
21CFR346.14	ANORECTAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE Active Ingredients Protectant active ingredients. (a) The following active ingredients may be used as the sole protectant active ingredient in a product if the ingredient as identified constitutes 50 percent or more by weight of the final product. In addition, the following active ingredients may be used in concentrations of less than 50 percent by weight only when used in combinations in accordance with 346.22 (a), (b), or (n). (3) Glycerin in a 20- to 45-percent (weight/weight) aqueous solution so that the final product contains not less than 10 and not more than 45 percent glycerin (weight/weight). Any combination product containing glycerin must contain at least this minimum amount of glycerin.
21CFR347.10	SKIN PROTECTANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE Active Ingredients Skin protectant active ingredients. The active ingredients of the product consist of any of the following, within the concentration specified for each ingredient: (h) Glycerin, 20 to 45 percent.
21CFR349.12	OPHTHALMIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE Active Ingredients The active ingredients of the product consist of any of the following, within the established concentrations for each ingredient: (d) Polyols, liquid: (1) Glycerin, 0.2 to 1 percent. demulcents.
	Agriculture
21CFR582.1320	SUBSTANCES GENERALLY RECOGNIZED AS SAFE General Purpose Food Additives (a)Product. Glycerin. (b)Conditions of use. This substance is generally recognized as safe when used in accordance with good manufacturing or feeding practice.
27CFR21.58	Alcohol, Tobacco Products and Firearms. CHAPTER I - ALCOHOL AND TOBACCO TAX AND TRADE BUREAU, DEPARTMENT OF THE TREASURY ALCOHOL. PART 21 - FORMULAS FOR DENATURED ALCOHOL AND RUM. Subpart D - Specially Denatured Spirits Formulas and Authorized Uses (a) Formula. To every 100 gallons of alcohol add: One hundred pounds of glycerin (glycerol), U.S.P., and 20 pounds of hard soap, N.F. XI. (b) Authorized uses. (1) As a solvent: 113.Lotions and creams (hands, face, and body). 131.Tooth paste and tooth powder. 141.Shampoos.
27CFR21.151	Title 27 - Alcohol, Tobacco Products and Firearms. CHAPTER I - ALCOHOL AND TOBACCO TAX AND TRADE BUREAU, DEPARTMENT OF THE TREASURY. SUBCHAPTER A - ALCOHOL. PART 21 - FORMULAS FOR DENATURED ALCOHOL AND RUM. Subpart G - Denaturants Authorized for Denatured Spirits. List of denaturants authorized for denatured spirits.Context: Glycerin (Glycerol), U.S.P; Specially Denatured Alcohol. 31-A.
40CFR180.1250	C8, C10, and C12 fatty acid monoesters of glycerol and propylene glycol; exemption from the requirement of a tolerance. The C8, C10, and C12 straight-chain fatty acid monoesters of glycerol (glycerol monocaprylate, glycerol monocaprate, and glycerol monolaurate) and propylene glycol (propylene glycol monocaprylate, propylene glycol monocaprate, and propylene glycol monolaurate) are exempt from the requirement of a tolerance in or on all food commodities when used in accordance with approved label rates and good agricultural practice.
40CFR180.910	Inert ingredients used pre- and post-harvest; exemptions from the requirement of a tolerance. Residues of the following materials are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest: [various glycerin-containing compounds]
40CFR180.920	Inert ingredients used pre-harvest; exemptions from the requirement of a tolerance. The following materials are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only: Glycerol—propylene oxide polymer (CAS Reg. No. 25791-96-2); Component in water-soluble film
40CFR180.930	Inert ingredients applied to animals; exemptions from the requirement of a tolerance. The following materials are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals: Glycerol monooleate; Surfactants, related adjuvants of surfactants Glyceryl monostearate; Emulsifier
40CFR180.950	Tolerance exemptions for minimal risk active and inert ingredients. Unless specifically excluded, residues resulting from the use of the following substances as either an inert or an active ingredient in a pesticide chemical formulation, including antimicrobial pesticide chemicals, are exempted from the requirement of a tolerance under FFDCA section 408, if such use is in accordance with good agricultural or manufacturing practices. (e) Specific chemical substances. Residues resulting from the use of the following substances as either an inert or an active ingredient in a pesticide chemical formulation, including antimicrobial pesticide chemicals, are exempted from the requirement of a tolerance under FFDCA section 408, if such use is in accordance with good agricultural or manufacturing practices. Glycerol (glycerin) (1,2,3-propanetriol)

Table 4. Acute toxicity studies of glycerin

Animal (n, if provided)	Results and notes	Reference
	Oral	
Rat	LD_{50}= >10000 mg/kg	2
Rat	LD_{50}=12600 mg/kg	2
Long-Evans, rat, female (12)	LD_{50}=27200 mg/kg for both natural and synthetic glycerin. Purity of both test materials = 99.5% administered neat. Clinical signs included muscle spasms and convulsions. Survivors appeared normal within 2.5 h of dosing. Number of deaths was not reported. Macroscopic examination of decedents and survivors showed hyperemia of the pylorus, small intestine and cerebral meninges (3 rats), and congestion of the lungs and pale spleen.	17
Sprague-Dawley rat (10)	LD_{50}=>2530 mg/kg	18
Fischer 344 rat, female (5)	LD_{50}=>24000 mg/kg Glycerin/water mixture of unknown composition. No deaths at 48 h.	19
Wistar rat, male (10)	LD_{50}=27500 mg/kg	20
Rat	LD_{50}=>25000	14
Rat	LD_{50}=58400	21
NMRI mouse, male and female (10)	LD_{50}=37950	18
Mouse	LD_{50}= 4090 mg/kg	2
Mouse	LD_{50}~26000 mg/kg. LD_{50} for natural glycerin = 20.65 cc/kg; LD_{50} for synthetic glycerin = 20.81 cc/kg. Purity of the synthetic glycerin = 99.8%.	22
Mouse	LD_{50} = 38000 mg/kg	18
Swiss mouse, male	LD_{50}=23000 for both natural and synthetic glycerin. Purity of both test materials = 99.5% administered neat. Body tremors, erection of the tail, and generalized clonic convulsions preceded all observed deaths of mice.	17
Mouse	LD_{50}=4250 mg/kg	2
Mouse	LD_{50}=>38000	14
Mouse	LD_{50}=37763	21
Mouse	LD_{50}=25888 mg/kg	21
Mouse	LD_{50}=12500 mg/kg	23
Mouse	LD_{50}=25000 mg/kg	23
Rabbit	LD_{50} = 27000 mg/kg	2
Guinea pig (9-10)	LD_{50}=10000 ± 130 mg/kg for natural glycerin and 11500 ± 2800 mg/kg for synthetic glycerin.. Purity of both test materials = 99.5%; administered neat. Tremors of the head and body, initiated by auditory stimuli, occurred immediately after injection. Death was usually preceded by tremors, but not all guinea pigs with tremors died.	17
Guinea pigs (10)	LD_{50}=77500 mg/kg	20
	Dermal	
Rat	LD_{50} = > 21900 mg/kg. 2.52g of the neat liquid (21900 mg/kg) for more than 20 min produced excretion of hemoglobin in the urine of male rats, indicating red blood cell damage. No deaths.	2
Rabbit (6)	LD_{50} = > 18700 mg/kg for both natural and synthetic glycerin. Purity for both natural and synthetic glycerin = 99.5%. Glycerin under occlusion for 8 h. No clinical signs were observed for either synthetic or natural glycerol.	17
	Intraperitoneal	
Rat	LD_{50} = 7500 – 10100 mg/kg	2
Rat	LD_{50} = 4420 mg/kg	2
Mouse	LD_{50} = 8600 – 9500 mg/kg	2
Mouse	LD_{50} = 8700 mg/kg	2
	Subcutaneous	
Rat	LD_{50} = 100 mg/kg	2
Mouse	LD_{50} = 91 mg/kg	2
Mouse	LD_{50}=10000 mg/kg	23
	Intravenous	
Rat	LD_{50} = 5200 – 6600 mg/kg	2
Rat	LD_{50} = 5566 mg/kg	2
Mouse	LD_{50} = 5700 – 6700 mg/kg	2
Mouse	LD_{50} = 4250 – 4370 mg/kg. LD_{50} for natural glycerin = 4.37 g/kg; LD_{50} for synthetic glycerin = 4.25 g/kg. Purity of the synthetic glycerin = 99.8%.	2
Mouse	LD_{50} = 4250 mg/kg	2
Mouse	LD_{50}=6000 mg/kg	23
Rabbit	LD_{50} = 53000 mg/kg	2

Table 5. Repeated dose toxicity studies.

Animal	n	Results and notes	References
		Oral	
Charles River rat, female	10, 20 control	LOAEL = 950 mg/kg. 0, 0.75, 1.5, or 3.0 mg/kg glycerin (950, 1900 and 3800 mg/kg bw); 100% by stomach tube; 3 times/day for 3 days. Undiluted glycerin caused a dose-dependent increase in the number of animals showing hyperemia, petechial hemorrhage and erosions was seen. Dilution of glycerin reduced the effects.	24
Wistar rats, male	24, 18 controls. 3 control and 4 treatment rats were killed and necropsied at 6 times	Glycerin replaced the 53.4% carbohydrate in feed for 20 d. Controls had stock carbohydrate or were fed a stock diet calculated to deliver the same calories as the glycerin diet. Controls gained weight at 6%/d, glycerin fed and pair-fed controls gained 3%/d. Livers were 6% bw in the glycerin-fed rats and 4.6% in the other two groups. Kidneys of the glycerin-fed rats were 45% heavier than normal; the fat pad was normal. Enzymes increased and remained high in activity in the kidneys and livers of the glycerin-fed rats. At the end of the experiment, 95% of the ingested glycerin was being retained and blood glucose and liver glycogen were above normal. Water intake and urine production in the glycerol-fed rats were ~ 5 times higher than the normal.	25
Carworth rat, male	Not specified	No adverse effects. Diet containing 20% glycerin (8824 mg/kg bw/d) for 4 weeks. At the end of 4 weeks, 5 rats were killed and necropsied. Both epididymus fat pads were excised, dried, and weighed. Liver total lipids and cholesterol were determined.	26
Rat, male	20	NOAEL between 115 and 2300 mg/kg. 0, 1%, 5%, 10%, 20% (115, 575, 1150 and 2300 mg/kg) in water; 1 mL; for 44 days. No adverse effects were observed for growth curves, lethality, and histological examination of the kidneys, livers, and bladders. Mortality was 15% in all groups.	27
Rat, female	5	5% natural and synthetic glycerin in drinking water (3335 mg/kg/day) for 6 months. No effects on growth, red blood cells and hemoglobin. Macroscopic incidental findings were a small thymus in 2 rats and slight interstitial pneumonia in one on natural glycerin and small spleen (with small lymph nodes and moderate hemosiderin deposits) and thymus atrophia in one animal that died on synthetic glycerol. Calcified masses in kidney tubulus between cortex and medulla in 3/5 rats on natural glycerin and 3/5 rats on synthetic glycerin.	22
Long-Evans rat, male and female (22/sex; 26 controls)	6-7	NOAEL=10 000 mg/kg based on the absence of treatment-related effects in the high-dose group. Diet containing 0, 5%, 10%, or 20% natural or synthetic glycerin for 2 years. Purity 99.5%. Feed consumption was increased in males at 5% and 10% natural glycerin. No treatment related effects in hematology, urinalysis, albumin, organ weights, gross pathology, and liver glycogen and lipids. Incidental bronchiectasis, pneumonia, pulmonary abscesses, *taenia* infestation of the liver, hydronephrosis and pyelonephritis (27 rats total).	17
Rabbit	4	No adverse effects. 0 or 50%, 10 mL in saline or saline by stomach tube or from a drinking cup daily for 30-40 d. Well tolerated. Necropsy at the end of the experiment showed no gross pathological changes. Neither the plasma nor the red blood cell cholesterol levels showed any consistent changes which could be attributed to glycerin.	29
Mongrel dog, male and female	Not specified	NOEL=950 mg/kg. 950, 1900 and 3800 mg/kg 3 times/d for 3 days. At 950 mg/kg bw: no abnormalities. At 1900 mg/kg: stomach mucosa was severely hyperemic with petechial hemorrhages. At 3800 mg/kg: stomach mucosa was (slightly to) severly hyperemic with areas with petechial hemorrhages or erosions; duodenum appeared normal or with hyperemic areas.	24
Dog	Not specified	0, 35% in feed for 50 weeks, then reduced to 50%-80% of previous dose. Body weight similar between groups until week 36 then after week 36 weight loss (16%, 1.8 kg) in dogs on glycerin rich diet but not in controls. Erythrocyte counts were similar between groups.	28
Guinea pig	10	0 or 50% in saline (= 6300 mg/kg/day) by stomach tube or from a drinking cup daily for 30–40 d. Guinea pigs administered > 5 ml of the 50% glycerin solution by stomach tube died with acute symptoms. Necropsies revealed no pathological changes. Plasma cholesterol levels had no changes attributable to glycerin. Red blood count of 3 guinea pigs (2 stomach tube, 1 drinking water) indicated a probable anemic effect.	29
		Dermal	
Rabbit	12	No treatment related effects at 100% after 90 days of administration of 0.5-4 mL of both natural and synthetic glycerin administered to 30% of the body surface for 8 h/d 5 d/wk, 45 weeks. Purity of both = 99.5%.	17
		Inhalation	
Sprague-Dawley Crl:CD Rat,, male/female	10/sex	LOAEL=1000 mg/m^3 based on local effects on the epithelium of the upper respiratory tract. 0, 1000, 2000, 4000 mg/m^3 for 6 h/d, 5 d/wk for 2 weeks. Nose only exposure. Particle size=<1.5µm. 2 males at 1000 mg/m^3 and 1 male and 1 female at 2000 mg/m^3 died. No clinical signs observed. Body weight gains were decreased in males and females at all concentrations (28%-58% in females). Glucose decreased in females at all concentrations (19%-28%). No treatment related effects for hematology, organ weights, and gross pathology. Histopathology: minimal to mild squamous metaplasia of the epiglottis in males and females (1/10, 13/18, 16/19, and 13/14, respectively). No dose-related increase in the frequency, but the incidence of mild metaplasia was greatest in the high-dose (7 animals with minimal and 6 with mild).	22,30
Sprague-Dawley Crl:DCD rat, male/female	15/sex	NOAEL = 0.167 mg/L. 0, 0.033, 0.167, 0.662 mg/L for 5 h/d, 5 d/week, 13 weeks. purity >99.8%, particle size <2.0 µm Nose–only study. Minimal to mild squamous metaplasia of the epithelium lining the base of the epiglottis. 3/sex necropsied at 10 and 13 weeks to examine lungs with electron microscope. No clinical signs or mortalities. No treatment related effects for body weights, clinical chemistry,	22

	hematology, organ weights, and gross pathology. Histopathology: minimal squamous metaplasia of the epiglottis in 2/25, 1/19, 4/20 and 10/21 rats, respectively; 1 male at 662 mg/m^3 showed mild squamous metaplasia. No differences in morphology of the Clara cells in control and high dose rats and histopathology.	

Table 6. Fertility studies of glycerin in males.

Test animal (n)	Concentration; route	Results; notes	Reference
Sprague-Dawley rat; age 48, 69, 90-95 days old (12)	0, 50-200 µL; 2 intratesticular injections 7 days apart into right testes; left was control	Testis treated with 50 µL decreased in weight (45%-60% within 2 weeks) compared to control side for all ages and complete loss of spermatogenic cells. Testis treated with 200 µL had decreased weights of prostate and seminal vesicles over 73 d. Number of sperm/epididymis declined rapidly, reduced by 99.99% (of controls) after the 3rd mating. Females were added in weeks 2, 3, 4, 5 and 6. Treated males mated with virgin females at same frequency as controls but all were infertile after 3rd mating and remained infertile for the duration of the tests (21 weeks after treatment). No resumption of spermatogenesis	35
Rat (np)	862 mg/kg; Intratesticular injection 1 day prior to mating	Suppressed spermatogenesis (meiosis). No evidence of toxic or endocrine effects.	33
Monkey	119 mg/kg; Intratesticular injection 1 day prior to mating	Suppressed spermatogenesis (meiosis). No evidence of toxic or endocrine effects.	34
Human (64; control, 63)	Exposure through working in a factory manufacturing glycerin	No differences observed sperm counts and percent normal forms compared with a control group.	36

Table 7. Genotoxicity assays of glycerin.

Assay	Concentration	Result; comments	Reference
		In Vitro	
Ames test using *S. typhimurium* (strain TA100)	0.1 and 1 mmol/plate	Negative with and without metabolic activation	40
Ames test using .*S typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538)	0.2, 0.4, 0.6, 0.8, and 1.0 mg/plate	Negative with and without metabolic activation	37
Ames test using .*S typhimurium* (strains TA98, TA100, TA1535, and TA1537)	10 mg/plate	Negative with and without metabolic activation; tested in 3 laboratories. One lab had ambiguous results.	38
Ames test using .*S typhimurium* (strains TA98, TA100, TA1537, and TA1538)	10 mg/plate	Negative with and without metabolic activation	19
Ames test using *S. typhimurium* (strain TA100)	0.5 mg/plate	Negative with and without metabolic activation	41
Ames test using *S. typhimurium* (strains TA94, TA98, TA100, TA1535, and TA1537)	50 mg/plate	Negative with and without metabolic activation	39
Ames test using .*S typhimurium* (strains TA98, TA100, TA1535, and TA1537)	1 -10 µg/plate; Glycerin/water mixture of unknown composition	Negative with and without metabolic activation	19
Cytogenetic Assay using CHO cell line WBL	0.1, 0.2, 0.3, 0.6, 0.8, and 1.0 mg/mL	Negative with and without metabolic activation	37
HGPRT assay using CHO (K1 and BH4 cell lines)	0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL	Negative with and without metabolic activation	37
Sister chromatid exchange assay using CHO (cell line WBL)	0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL	Negative with and without metabolic activation; purity >99.5%.	37
Unscheduled DNA synthesis using rat hepatocytes	0.1, 0.25, 0.5, 0.75, and 1.0 mg/mL	Negative without metabolic activation; purity >99.5%.	37
Chromosomal aberration test using CHO cells	1 mg/mL	Negative 100 metaphases analyzed.	39

Table 7. Genotoxicity assays of glycerin.

Assay	Concentration	Result; comments	Reference
		In Vivo	
Chromosome aberration assay using male rats (species and number not specified)	1 mg/kg; orally in water or saline administration	Number of cells with aberrations 2.2% vs. 0% in concurrent controls; cells with gaps 1.6% vs. 0%; polyploid cells 3.2% vs. 0%. Purity of the test substance is not specified.	42
Chromosome aberration assay using male rats	1 g/kg by injection into abdomen	Negative Cytogenic analysis was performed in 50 metaphases	42
Dominant lethal gene using rats, male/female	0, 10, 100 and 1000 mg/kg	Male rats (number not specifed) were probably injected in the abdomen then 2 weeks after mating females were killed and necropsied. Trend for potential mutagenic effect on gender cells, resulting in post-implantation deaths but did not reach statistical significance. Implantation sites: 116, 101, 104, respectively. Fetal loss: 8%, 11%, 20%, and 59%, respectively. Live fetuses: 107, 90, 83 and 37, respectively. No anomalies observed in treatment and control groups.	42

The CHO/HGPRT assay detects forward mutations of the locus (coding for the enzyme, HGPRT) in Chinese hamster ovary (CHO) cells.

Table 8. Carcinogenicity studies of glycerin.

Test animal (n)	Concentration and administration route	Result; comments	Reference
Male and female rats (strain not specified; 24)	5 or 10 g/kg in feed for 2 yr	No increase in the incidence of tumors	17
Male and female Long-Evans rats (22/sex)	0, 5%, 10%, in diet for 2 yr; 20% in diet for 1 yr; natural and synthetic glycerin	No increased incidence of tumors following treatment with glycerin. Body weight gain: no differences between treatment and control groups. Histopathology: malignant neoplasms in 5/26, 1/22, 5/22, 0/22, 0/21, 5/22 and 0/22 rats for natural glycerol and synthetic glycerin, respectively. Benign neoplasms in 0/26, 2/22, 1/22, 0/22, 4/21, 4/22 and 1/22, respectively. Among the benign tumors 3 rats were found with pheochromocytomas, 2 with granulosa cell tumors.	17
		Synergistic effects	
ddy Mouse (18-20)	0 or 5% in drinking water for 1–4 weeks	Increased number of pulmonary tumor–bearing mice and mean number of induced tumors/mouse in mice administered glycerin for 4–25 weeks after 4NQO treatment, compared with mice given 4NQO alone. No. of mice with tumors: controls (no 4NQO)-1/20; controls (4NQO)-8/20; 1 week glycerin-11/20; 2 weeks glycerin-11/19; 3 weeks glycerin-7/18; 4 weeks glycerin-15/19	45
Male ddy mice (n = 20)	0, 5% (~8350 mg/kg/d) in drinking water for 25 weeks with and without a single injection of 4NQO	Glycerin alone did not result in an increase in number of mice with tumors compared to untreated controls. Glycerin did have a synergistic effect with 4NQO. 2 rats died (weeks 25-28) with only fibrosarcomas at injection site, only these had these tumors. Body weight: no treatment related effects. Pulmonary tumors: No. of mice with tumors: controls-2/20; controls (glycerin)-2/20; treatment (4NQO)-5/20; treatment (4NQO + glycerin) -17/20. Mean number of tumors/mouse: increased after 4NQO + glycerin-2.9/mouse vs. 0.1-0.45/mouse in the other groups. Histopathology: 4NQO treated mice all tumors were identified as type II adenomas. In 4NQO + glycerin treated mice 52 tumors were identified as type II adenomas and 6 as Clara cell adenomas.	43
Male ddy mice (n = 10)	0, 5% (~8350 mg/kg/d) in drinking water for 25 weeks with and without a single injection of 4NQO	Glycerin promoted tumorgenesis when administered after 4NOQ. No. of mice with tumors: controls 0/10; controls (glycerin)-0/10; controls (4NQO)-1/10; treatment (4 weeks glycerin)-8/10; treatment (25 weeks glycerin)-8/9; treatment (glycerin week 4-25)- 7/10. - Mean number of tumors/mouse: controls-0; controls (glycerin 25 weeks)-0; controls (4NQO)-0.1; treatment (4 weeks glycerin)-3.5; treatment (25 weeks glycerin)-2.3; treatment (glycerin week 4-25)-1.9. Histopathology: All tumors were adenomas.	44

4NQO – 4-Nitroquinoline 1-oxide

Table 9. Dermal irritation studies.

Animal (n, if provided)	Results and notes	Reference
Rabbit (12)	Not irritation at 100% after 90 days of administration. Draize test of 0.5-4 mL of both natural and synthetic glycerin administered to 30% of the body surface for 8 h/d 5 d/wk, 45 weeks. Purity of both = 99.5%.	17
Rabbit, New Zealand female (6)	Not irritating 0.5 mL glycerin/water mixture of unknown composition. Draize scale score 0.1 for intact and abraded skin.	19
Rabbit, albino male (8)	Not irritating 0.5 mL administered to 6.25 cm^2 of skin for 24 hours. No signs of irritation at 24 and 72 h. Draize scale scores 0-0.4 compared to a maximum score of 30.	46
Guinea pig (~45)	Mildly irritating; + 0.1 cc administered to the shaved abdominal skin and observed at 4 and 24 h.	23

Table 10. Ocular irritation studies.

Animal (n)	Results and notes	Reference
Rabbit (6)	Not irritating 0.1 mL at 100%. No irritation at 1, 24 and 72 h and 7 days. Overall Draize score 0-2 on a scale of 110.	46
Rabbit (4)	Not irritating for both natural and synthetic glycerin with purity of 99.5%. The conjunctiva was irritated in all rabbits 1 h after treatment. Resolved at 24 h after treatment.	17
Rabbit, New Zealand White, female (6)	Not irritating 0.1 mL at 100% glycerin/water mixture of unknown composition. Overall Draize score 0.4 at 1h, 0 at 24-96 h.	19
Rabbit (5)	Mildly irritating; + for both edema and hyperemia. ~0.5 cc at 100%	23

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